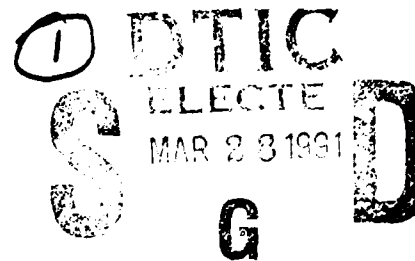


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## VISCERAL LEISHMANIASIS UNRESPONSIVE TO PENTOSTAM CAUSED BY *LEISHMANIA TROPICA* IN KENYA

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**Abstract.** We report the characterization of 6 *Leishmania tropica* isolates from 2 patients with visceral leishmaniasis who were unresponsive to treatment with sodium stibogluconate. The *Leishmania* isolates, MHOM/KE/81/NLB-029A, -029X1B, and -029XIC and MHOM/KE/81/NLB-030I, -030B, and -030XXA, all from splenic aspirates, were characterized by cellulose acetate electrophoresis using 11 enzymes: malate dehydrogenase, malic enzyme, phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, superoxide dismutase, glutamate-oxaloacetate transaminase, adenylate kinase, nucleoside hydrolase, mannose phosphate isomerase, glucose phosphate isomerase, and phosphoglucomutase. Isozyme migration patterns were indistinguishable from those of 2 WHO reference strains of *Leishmania tropica* (MHOM/SU/60/LRC-L39, NLB-305 and MHOM/IQ/OO/LRC-L36, NLB-067). These are the first reported cases of visceral leishmaniasis (kala-azar) caused by *L. tropica* in Africa; these cases were refractory to sodium stibogluconate.

A third of the world's population, about 1,600 million people, is at risk of infection and disease due to the leishmaniasis.<sup>1</sup> Precise prevalence and incidence data are not known.<sup>1</sup> An approximate incidence rate of 400,000 new cases of leishmaniasis per year has been reported.<sup>1-2</sup> The different species and sub-species of the genus *Leishmania* produce varying manifestations in man.<sup>3</sup> At 1 end of the spectrum lies the papular-ulcerative, self-healing cutaneous leishmaniasis; the often fatal visceral leishmaniasis (kala-azar) is at the opposite end. Somewhere in the middle lies the erosive mucocutaneous form and the rare diffuse cutaneous form, which rarely, if ever, achieve a spontaneous cure.<sup>3</sup>

Sodium stibogluconate (Sbv/Pentostam®) is the drug of choice for the treatment of responsive (sensitive) leishmaniasis in most of its manifestations. However, not all patients with any form of leishmaniasis respond to treatment with Sbv. Mucocutaneous and diffuse cutaneous leishmaniasis are commonly antimony resistant.<sup>3-5</sup>

In Kenya, both cutaneous and visceral leishmaniasis have been reported since the early 1940s.<sup>6</sup> The treatment of choice for visceral leishmaniasis in Kenya since the early 1960s has

been a 30-day course of Sbv at 10 mg/kg/day.<sup>7</sup> Because of a 21% relapse rate with the dosage regimen,<sup>7,8</sup> daily Sbv dosages of 20 mg/kg for 30 days have been used in Kenya. Rees and others<sup>9</sup> have pointed out that with this daily dosage of Sbv, kala-azar cure rates of >90% can be achieved in most countries with visceral leishmaniasis. Treatment doses and the duration of treatment vary.<sup>3,9-12</sup>

*Leishmania tropica*, the causative agent of urban cutaneous leishmaniasis in the Old World, has been isolated from a small number of patients with visceral leishmaniasis.<sup>13,14</sup> *L. infantum*, which usually causes infantile visceral leishmaniasis, has been isolated from cutaneous lesions.<sup>15</sup> A recent finding was reported from Brazil of *L. mexicana amazonensis* from the bone marrow of a patient having American visceral leishmaniasis.<sup>16</sup> This parasite is thought to cause human American cutaneous leishmaniasis, which most often progresses into diffuse cutaneous leishmaniasis.<sup>17</sup>

We report the characterization of *L. tropica* isolates from 2 patients with visceral leishmaniasis who were unresponsive to treatment with sodium stibogluconate.

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## CASE HISTORIES

*Case 1: isolates NLB-029A, -029XIB, and -029XIC*

A 2-year-old Kamba girl from Kikumini, Masinga location, Machakos District was admitted on 24 January 1981 to the Clinical Research Center (CRC) with a 6 month history of abdominal swelling, fever, and malaise. Examination revealed splenomegaly in a poorly nourished child. A splenic aspirate done on admission showed a 6+ parasite load.<sup>10</sup> She was treated with sodium stibogluconate (Sbv) 10 mg/kg given every 12 hr from 29 January 1981 until 12 February 1981 with no improvement; the parasite count remained unchanged. The Sbv dosage was increased to 20 mg/kg daily from 20 February 1981 until 22 May 1981. The parasite count dropped from 4+ to 1+, and the patient remained in the hospital for further observation until 6 July 1981, when she was discharged. At this time, the spleen was not palpable and splenic aspirates were found negative for *Leishmania* by smear and culture.

The patient was re-admitted on 25 August 1981. Splenic aspirate showed a parasite count of 5+ for *Leishmania* and she was given 20 mg/kg daily Sbv from 12 September 1981 until 3 November 1981. The spleen remained enlarged. Smears showed an initial parasite count of 1+, but this later increased to a 4+ parasite count. The patient was then treated with pentamidine 4 mg/kg 3 times/week for 18 weeks, and then the dose was increased to 5 mg/kg 3 times/week for another 7 weeks. At the same time, the child received allopurinol 20 mg/kg/day for 17 weeks. On 24 May 1982, the spleen was non-palpable but intercostal aspirate was 2+ by smear. Subsequent smears made on 28 June, 9 August, and 30 August 1982 were all negative.<sup>11</sup> After 5 April 1982, the course was complicated by abscesses at the pentamidine injection sites, which eventually healed. At the time of discharge, the patient had depressed scars with clean bases.

The patient returned on 29 November 1982 to the CRC and was found negative for *Leishmania* by smear and culture. She was seen for subsequent follow-up visits for 1 year and did not relapse.<sup>11, 12</sup>

*Case 2: isolates NLB-030I, -030B, and -030XXA*

A 9-year-old Kamba boy from Kikumini, Masinga location, Machakos District, was ad-

mitted to the Infectious Diseases Hospital on 17 January 1981. The patient was the brother of the first case.<sup>10-12</sup> Clinical examination revealed splenomegaly, and bone marrow smears showed *Leishmania* amastigotes. He was treated with IM Sbv, 10 mg/kg daily for 40 days (22 January 1981-22 February 1981 and 28 February 1981-9 March 1981) and then with 20 mg/kg given at 8 hr intervals for another 40 days (26 March 1981-2 May 1981). The patient did not show improvement. He was then treated with IM Glucantime, 20 mg/kg for 30 days (5 May 1981-6 June 1981). During this time, parasites persisted in the bone marrow. He was started on rifampicin treatment, 450 mg/day for 5 weeks (16 June 1981-21 July 1981). With no improvement in his condition, the patient was further treated with allopurinol (tablet form) 200 mg/day for 12 weeks. During this time, the patient's spleen decreased in size and his weight increased, but splenic aspirates continued to be positive. The patient was then started on a course of treatment with IV Sbv 20 mg/kg plus allopurinol 200 mg/day for 53 days (15 October 1981-7 December 1981).

Despite the persistence of parasites, it was decided to discharge the patient and re-admit him after 6 weeks. At discharge, the patient was clinically well with a slightly enlarged spleen. On re-admission, he was found to have an enlarged spleen and a parasite count of 5+ after splenic aspirate. He was put on rifampicin 600 mg/day plus allopurinol 200 mg/day for 6 weeks, but no improvement was noted either hematologically or in the parasite count. He was started on Sbv, 20 mg/kg 3 times/day for 18 days but still there was no improvement. He was then started on pentamidine 4 mg/kg IM once weekly after stopping the other medication. He received this regimen for 12 weeks, yet the number of parasites found in splenic smears increased. Pentamidine was increased to 4 mg/kg twice weekly for 24 weeks, and the parasite count gradually decreased until none were observed. His hemoglobin rose to 13 g% and the spleen size decreased considerably. Several cultures made from splenic aspirates and splenic smears on slides showed no parasites, and the patient was discharged.

## MATERIALS AND METHODS

Six *Leishmania* isolates (NLB-Q29A, -029XIB, -029XIC, and NLB-030XXA, -030I, and -030B) obtained from splenic aspirates of these 2 Kenyan kala-azar patients who had been treated

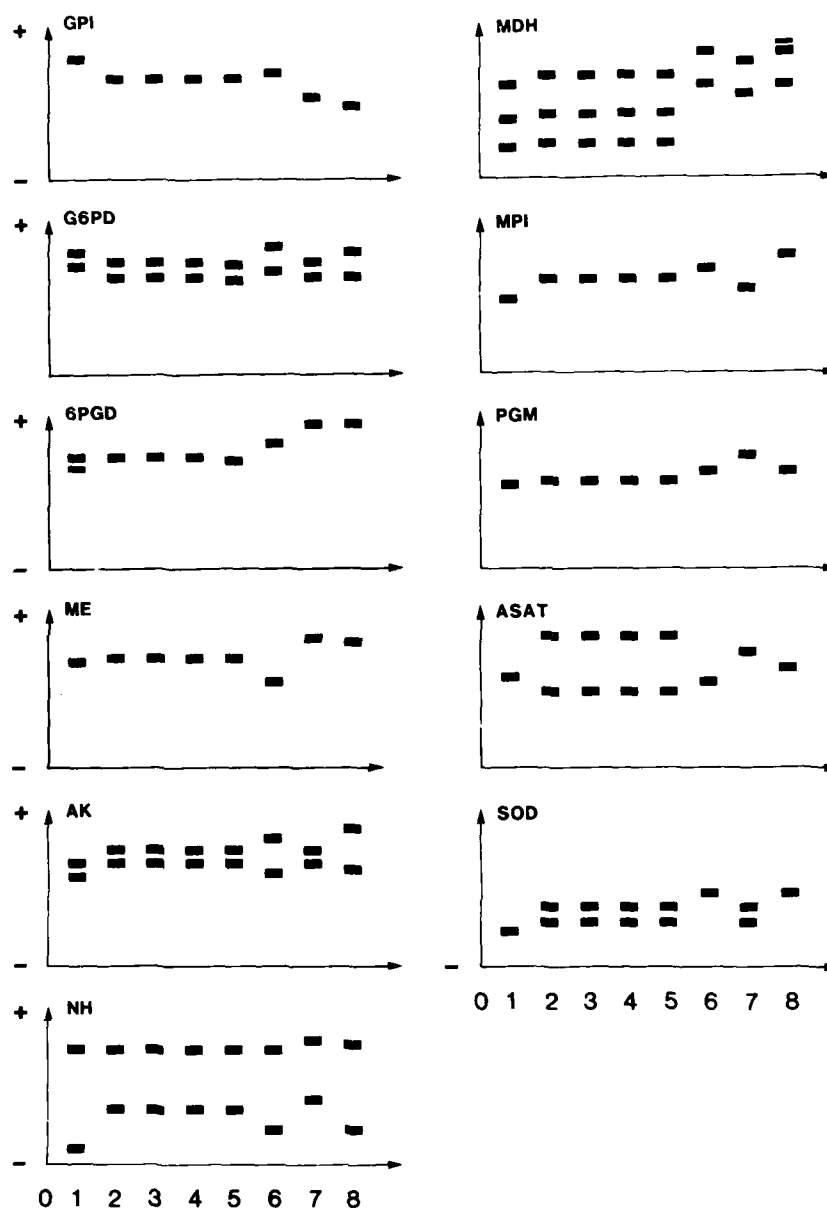


FIGURE 1. Banding patterns of 11 enzymes from Kenyan isolates, WHO reference strains, and other characterized strains.

- |                         |  |
|-------------------------|--|
| 1. <i>L. donovani</i>   | MHOM/ET/67/LRC-L133, NLB-061 (WHO) or<br>MHOM/KE/82/LRC-L445, NLB-065  |
| 2. <i>L. tropica</i>    | MHOM/SU/60/LRC-L39, NLB-305 (WHO)  |
| 3. Isolates-Case 1      | MHOM/KE/81/NLB-029A, 029XIB, 029XIC  |
| 4. Isolates-Case 2      | MHOM/KE/81/NLB-0301, 030B, 030XXA  |
| 5. <i>L. tropica</i>    | MHOM/IQ/00/LRC-L36, NLB-067 (WHO)  |
| 6. <i>L. major</i>      | MHOM/IL/67/JERICO-II, NLB-314=326 (WHO) or<br>MRHO/SU/59/P-STRAIN, LRC-L38, NLB-313 (WHO) or<br>IPHL/KE/83/LRC-L447, NLB-144 |
| 7. <i>L. aethiopica</i> | MHOM/ET/72/L100, LRC-L36, NLB-310 (WHO) or<br>MHOM/KE/70/LV-266, NLB-007   |
| 8. <i>L. arabica</i>    | MHOM/SA/83/JISH-220, NLB-664   |

with Pentostam® or other anti-leishmanial drugs were inoculated into 25 cm<sup>2</sup> ml culture flasks (Corning®, Corning, NY) with 10 ml Schneider's *Drosophila* Medium (GIBCO, Grand Island, NY), supplemented with 20% heat-inactivated fetal bovine serum plus penicillin (250 U/ml), streptomycin (250 µg/ml), gentamicin (260 µg/ml) and 5-fluorocytosine (500 µg/ml). After inoculation, the cultures were incubated at 25°C for 6–7 days to allow growth to a stationary phase, after which lysates of the promastigotes were made for use in electrophoresis assays.

Electrophoresis was performed according to the methods of Kreutzer and Christensen<sup>18</sup> and Kreutzer and others<sup>19</sup> for all enzymes except 3, MDH, NH, and SOD, which were assayed according to Le Blancq and others<sup>20</sup> and Le Blancq and Peters.<sup>21</sup> The 6 unknown leishmanial isolates, NLB-029A, -029X1B, and -029XIC, and NLB-030I, -030B, and -030XXA, were identified based on comparisons of their zymogram banding patterns with those of 10 reference strains (Fig. 1). The enzymes used in the assays were malate dehydrogenase (MDH) E.C. 1.1.1.37, malic enzyme (ME) E.C. 1.1.1.40, phosphoglucuronate dehydrogenase (6PGD) E.C. 1.1.1.44, glucose-6-phosphate dehydrogenase (G6PD) E.C. 1.1.1.49, superoxide dismutase (SOD) E.C. 1.15.1.1, glutamate-oxaloacetate transaminase (GOT/ASAT) E.C. 2.6.1.1, adenylate kinase (AK) E.C. 2.7.4.3, nucleoside hydrolase (NH) E.C. 3.2.2.2, mannose phosphate isomerase (MPI) E.C. 5.3.1.8, glucose phosphate isomerase (GPI) E.C. 5.3.1.9, and phosphoglucomutase (PGM) E.C. 5.4.2.2.

## RESULTS

The 6 *Leishmania* isolates from the 2 indigenous Kenyan kala-azar patients had 11 of 11 enzyme profiles that differed from the reference strains of *L. donovani*, *L. major*, and *L. arabica* (Fig. 1). Two *L. aethiopica* reference strains had isozyme patterns similar to those of the patient isolates, and to the 2 *L. tropica* markers for G6PD, SOD, and AK (Fig. 1). Except for the WHO reference strain of *L. tropica* (NLB-067), which had a slightly lower 6PGD and G6PD isozyme migrating bands, the isolates from the 2 Kenyans had 11 electrophoretic isozyme patterns which were indistinguishable from those of the 2 *L. tropica* reference strains (Fig. 1).

## DISCUSSION

This is the first report of human visceral leishmaniasis caused by *L. tropica* in Africa. Reports have implicated *L. tropica* as a causative agent of visceral leishmaniasis in the Middle East and in the Indian subcontinent.<sup>13,14,22,23</sup> Schnur and others<sup>13</sup> characterized 5 *Leishmania* strains from visceral cases, 4 from Israel and 1 from India, against 3 known reference strains of *L. tropica* from Iraq, Turkestan (USSR), and India. All 5 strains were indistinguishable from the reference strains of *L. tropica*, biochemically and serologically. Schnur felt these findings were suspect because most of the isolates had been transferred from 1 laboratory to another, increasing the possibility of contamination with other isolates. Recently, Schnur and Oren reported with certainty 1 human case of kala-azar caused by *L. tropica* in Israel (L. F. Schnur, Kuvim Centre, Jerusalem, personal communication). According to Lainson,<sup>24</sup> *L. tropica* causes leishmaniasis recidivans, with long-lasting multiple lesions, and on rare occasions has given rise to kala-azar.

One important clinical aspect of the cases of human viscerotropic *L. tropica* in Kenya is that they were refractory to sodium stibogluconate treatment alone, but responded to a combination of various anti-leishmanial drugs administered for a prolonged period of time. The 2 patients had several relapses and had to be treated with various anti-leishmanial drugs for a long time before responding favorably.<sup>11,12</sup> Because these patients were siblings, Bryceson and others have suggested the possible existence of genetic susceptibility or failure to respond to antimony, the possibility that patient 1 became infected with a "resistant parasite transmitted from her brother," and that unresponsive patients may not be able to metabolize pentavalent antimony normally.<sup>11,12</sup> Since *Leishmania* parasites were isolated after the 2 patients had been treated either with Pentostam® or other anti-leishmanial drugs, we acknowledge the possibility of selection of unresponsive parasites. We reported<sup>25</sup> that *L. tropica* causes cutaneous leishmaniasis in Kenya and also found 3 of these cases refractory to sodium stibogluconate treatment. These findings underscore the need for prompt determination of species and drug sensitivities in isolates from kala-azar patients. Identifying *Leishmania* species on the basis of clinical involvement in patients is unreliable.

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